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Customer No. 23308

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Inventor | : | Tabassum Naqvi | Art Unit. | : | 1641 |
| Serial Number | : | 10/689,122 | Examiner | : | HAQ, Shafiqul |
| Filed | : | 10/20/2003 | | : | |
| Title | : | $IP_3$ Protein Binding Assay | | : | |
| Confirmation Number | : | 4234 | Customer Number | : | 23308 |

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# DECLARATION UNDER 37 CFR §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Edwin F. Ullman, Ph.D., hereby declare as follows:

1. I am a consultant for the assignee of the subject application. My c.v. accompanies this declaration. I was asked to review the reference cited in the Official Action, Hirata, et

al., JBC (1990) 266, 8404, and provide my evaluation of the conclusions that may be reasonably drawn from the experimental work reported in the paper.

2. This paper describes the behavior of D-$IP_3$ and its 2-hydroxy ester derivatives, D- and L-206 and D- and L-209.

3. This paper shows the following:

Fig 2 shows the inhibition of $IP_3$-5-phosphatase activity in erythrocyte ghosts by the above compounds. The activity was determined by measuring the decrease in [$^3$H]$IP_3$. Only D-$IP_3$ and L-209 produce a significant decrease in the rate. This shows that the 2-substituted D-$IP_3$ derivatives do not bind at the active site of this enzyme.

Fig 4 shows the inhibition of $IP_3$ kinase activity in rat brain cytosol by the above compounds. The activity was determined by measuring the increase in [$^3$H]$IP_4$. Only D-$IP_3$, D-206 and D-209 were inhibitory. Whether the inhibition was actually due to binding of D-206 and D-209 to $IP_3$ kinase is questionable (see comments on Fig 5).

Fig 5 shows chromatograms of the mixtures obtained by reaction of each of the above compounds with [$^{32}$P]ATP in the presence of rat brain cytosol. Both D-$IP_3$ and D-206 were converted to [$^{32}$P]$IP_4$. This suggests that D-206 was first hydrolyzed to $IP_3$ by esterases which was then phosphorylated by an $IP_3$ kinase. Because kinases are usually highly specific it is less likely that D-206 could have first been phosphorylated and the product then hydrolyzed to $IP_4$. If the former is correct, the apparent Fig 4 conclusion that D-206 inhibits $IP_3$ kinase activity is not justified. It is more likely that the $IP_3$ formed from D-206 inhibited $IP_3$ kinase activity .

Fig 5 shows that D-209 was not converted to $IP_4$ or to any other unique ATP reaction product. It is therefore neither a substrate for the kinase or the esterases, and it is

possible that it is a true inhibitor of $IP_3$ kinase as suggested by Fig 4. Although less likely, it is also possible that its $IP_3$ kinase inhibitory activity arises from the product of some other reaction product such as dephosphorylation (see following paragraph), and that it too is not a true $IP_3$ kinase inhibitor.

The Fig 5 results are further complicated by the appearance of a $^{32}P$-labeled product (D- and L-isomers are not distinguishable) that elutes in 13 minutes and is common to all of the compounds. This requires that all four of the esters undergo hydrolysis, phosphorylation, and possibly dephosphorylation by enzymes that are present in rat brain cytosol. This observation further supports the likelihood that esterases play an important role in the kinase inhibition study.

Fig 6 appears to show that D-$IP_3$, D-206 and D-209, but not L-206 and L-209, can compete with [$^3$H]$IP_3$ for a binding site present in cerebellum microsomes. However this conclusion is not justified because of the likely possibility that the inhibition is due to esterase catalyzed hydrolysis of D-206 and D-209 to D-$IP_3$.

4. To summarize:

Binding of D-$IP_3$ to $IP_3$-5-phosphatase in erythrocyte ghosts is not inhibited by the 2-hydroxy esters, D-206 and D-209.

There is suggestive but non-rigorous evidence that D-209 binds to rat brain cytosol $IP_3$ kinase but there is no evidence that D-206 binds to this protein.

D- and L-206 and D- and L-209 or their phosphorylation or dephosphorylation products are substrates for rat brain cytosol esterases. There is no evidence as to whether one or more esterases are involved or the order of the steps leading to the common product.

D-206 and D-209 might compete with [$^3$H]D-$IP_3$ for binding to a protein in cerebellum microsomes, but the foregoing suggests that it is more likely that esterases in the microsomes convert one or both of these compounds to D-$IP_3$ which competes with [$^3$H]D-$IP_3$.

5. Conclusion

Because the experiments were carried out with different tissue preparations, each containing complex mixtures of binding proteins, this paper provides few definitive conclusions. The data suggest but do not prove that a single 2-hydroxy ester, D-209, binds to a rat brain cytosol $IP_3$ kinase. Apparent binding of the other 2-hydroxy ester, D-206, is likely due in whole or in part to formation of D-$IP_3$ upon esterase catalyzed hydrolysis of D-206. Moreover neither of these compounds bind to the $IP_3$ binding protein, $IP_3$-5-phosphatase, in erythrocyte ghosts, and the authors' conclusion that they bind to cerebellum microsomes proteins is unjustified. Accordingly there is no showing that 2-hydroxy D-$IP_3$ esters that bind to a particular $IP_3$ kinase binding site are able to bind other $IP_3$ kinases, and there is strong evidence that they cannot bind generally to any D-$IP_3$ binding protein.

6. Based upon these observations, it is my finding that one would have good reason to conclude from the paper that the 2-$IP_3$ derivatives would be unlikely to have affinity for the $IP_3$ receptor. Furthermore, in the subject application, the $IP_3$ receptor is not used for binding to the 2-derivative of $IP_3$, but rather a truncated receptor is used that has a much higher binding affinity. Therefore, neither does the reference support the conclusion that the $IP_3$ receptor would be expected to have a specific affinity for the 2-derivative of $IP_3$, nor does the subject application even use this protein. It would not have been obvious to expect that the 2-derivative of $IP_3$ would have a high affinity for the "sponge protein" derived from $IP_3$ receptor or for any other protein than possibly the $IP_3$ kinase from rat brain cytosol.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statement may jeopardize the validity of the application or any patent issued thereon.

Date: Jan 21, 2008 By: Edwin F. Ullman

Edwin F. Ullman, Ph. D.

Attachment: Curriculum Vitae

## CURRICULUM VITAE

**Edwin F. Ullman**
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## OCCUPATION

Consultant for biotech and biotech investment companies

## FIELDS OF EXPERTISE

SCIENCE AND TECHNOLOGY
- Immunochemistry and immunoassay methods
- Nucleic acid chemistry and assay methods
- Analytical methods and devices in clinical and medicinal chemistry
- Diagnostics and high throughput screening
- Chemistry of stable radicals as spin labels
- Organic photochemistry including fluorescent dye labels
- Organic and photochemical reaction mechanisms.

MANAGEMENT
- R&D Direction
- Corporate Strategies
- Patenting Strategies
- Expert Witness for patent litigation

## EMPLOYMENT

Research Chemist, Lederle Laboratories, American Cyanamid Company
Pearl River, New York, 1956-60

Group Leader, Central Research Division, American Cyanamid Company, Stamford, Connecticut, 1960-66

Scientific Director, Synvar Research Institute, Palo Alto, California, 1966-70

Vice President and Director of Research, Syva Company
Palo Alto, California, 1970-95

Vice President and Director of Research, Behring Diagnostics Inc.
San Jose, California, 1995-97

Chief Scientific Officer, ThauMDx, LLC, 2001-2

Chief Scientific Officern for Genomics and Advisor (part time), DiscoveRx Corp., 2002-2005

Advisor (part time), Innovations Research, 2004-2005

## EDUCATION

Reed College. Portland, Oregon, BA., 1952
Harvard University, Cambridge, Massachusetts, MS., 1954 Organic chemistry
Harvard University, Cambridge, Massachusetts, Ph.D., 1956 Organic chemistry

## AWARDS AND HONORARY POSITIONS

Phi Beta Kappa, 1952
National Science Foundation Fellow, 1952-3
Harvard Teaching Fellow, 1953-4
US Rubber Company Fellow, 1954-5
Clinical Ligand Assay Society, Mallinckrodt Award, 1981
Canadian Clinical Chemistry Society, MSD Health Group Award, 1982
New York Metropolitan Section of American Association for Clinical Chemistry Van Slyke Award, 1984
American Association for the Advancement of Science Fellow, 1987
Peninsula Patent Law Association Inventor-of-the-year Award, 1987
Northern California American Association of Clinical Chemists Outstanding Contributions to Clinical Chemistry Award, 1991
American Association for Clinical Chemistry, Award for Outstanding Contributions to Clinical Chemistry in a Selected Area of Research, 1997
The American Association for Clinical Chemistry and Dade Behring co-sponsor the Edwin F. Ullman Award given annually since 1998 by the American Association for Clinical Chemistry.

## EDITORIAL AND EDUCATIONAL ADVISORY BOARDS

Journal of Organic Chemistry Editorial Advisory Board, 1969-74
Journal of Immunoassay Editorial Board, 1979-
Journal of Clinical Laboratory Analysis Editorial Board, 1985-87
San Francisco State University Science & Engineering Advisory Board , 1994-96
Journal of the Clinical Ligand Assay Society, Editorial Board, 1999-01

**MEMBERSHIPS**

American Chemical Society
American Association of the Advancement of Science
American Association of Clinical Chemists

**PUBLICATIONS AND PATENTS**

Over 110 scientific publications and 230 issued US patents.